Evaluation of bioassays for surface water quality monitoring

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Mission: Source water Quality should allow drinking water production using simple treatment only!

- Initially “Pressure group” fighting water pollution
- Confronting polluters / decision makers with WQ data and demands
  - Strategy: actions based on sound science / hard evidence only!
  - Gradual shift from confrontation to cooperation
- International cooperation in IAWR

Research projects

- Emerging contaminants in STP effluents
  - EDCs, antibiotics, pharmaceuticals, XRFs,..
- Toxicity evaluation of emerging contaminants detected in inventory screenings
- Occurrence of cyanobacteria in surface waters and consequences for drinking water production
- Significance of anthropogenic organohalogens

WQ monitoring network

- Cooperation with Nat'l Dutch and German water authorities
  - Harmonized program (WQ variables, methods, data exchange,…)
- Five locations
  - German-Dutch border, intake sites
- Trend detection and compliance testing
  - Standardized freqs (13, 26)
  - Two types of WQ variables
    - "legal standards" & "emerging contaminants"
  - Chemical & biological

Why effect-oriented monitoring?

- Much more is out there than can be seen using regular chemical monitoring
- No info about the effects of the cocktail
  - EDCs, cholinesterase inhibition, genotoxicity,…
- Do such effects hamper ecosystem development / sustainability?
- Are such effects removed during drinking water treatment?

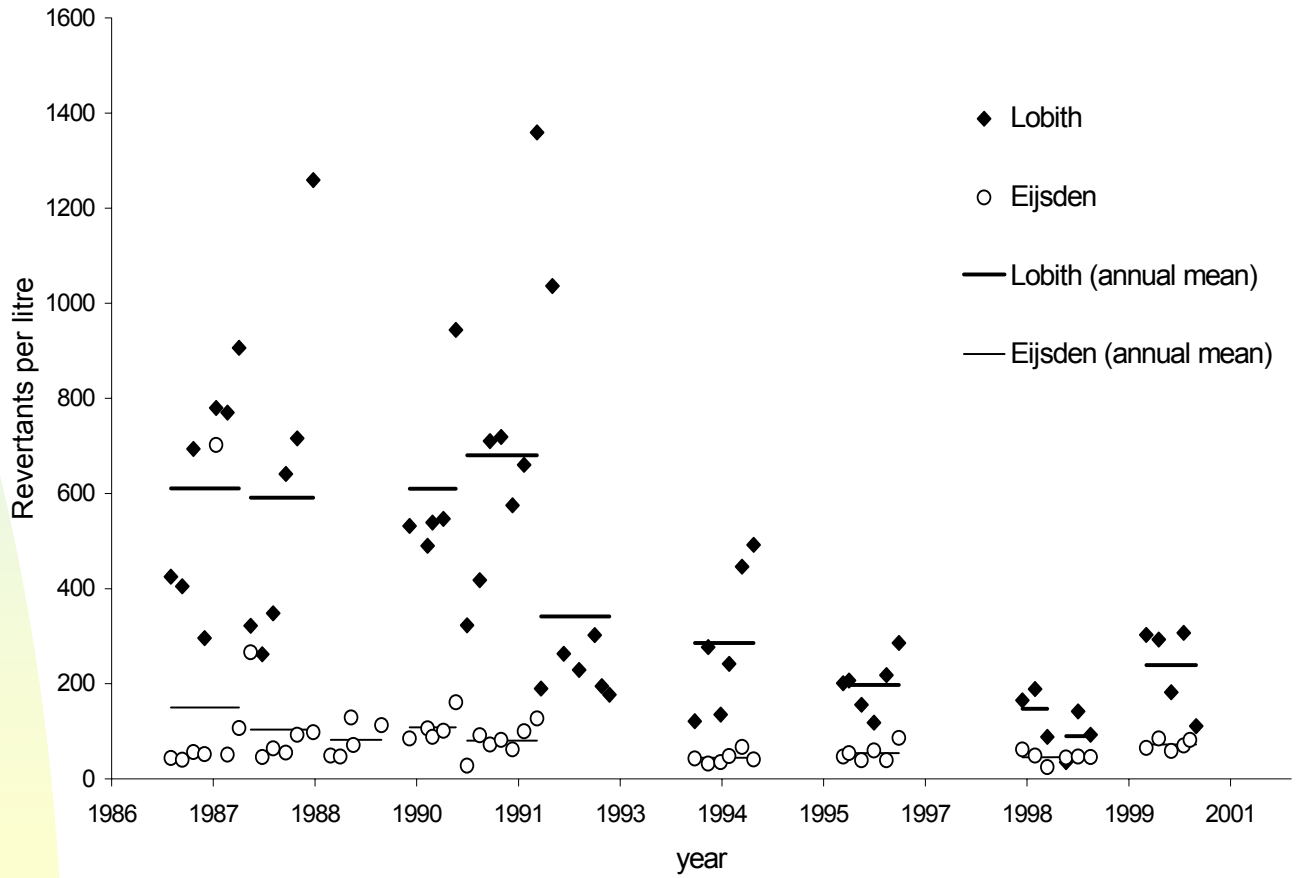
Ames TA genotoxicity test - 1

- Mutated strain of Salmonella typhimurium
- Does not grow on histidine-free culture medium
- Mutagenic substances may lead to reversal of mutation $\Rightarrow$regrowth (revertants)
- Widely used as test for presence of mutagenic substances in many different materials

Ames TA genotoxicity test - 2

- Included in RIWA WQMN since 1985
- Problem:
  - Cost ($10 - 20k/yr)
  - Disputed, notably upstream (pre-concentration step 25,000x)

Results from the Ames TA98 assay of Rhine and Meuse (only +S9, pH=7)



Evaluation of several effect-oriented tests

- Increased interest at Water authority level for effect-oriented approach in WQMNs
- Discussion about incorporation in discharge control
- Drawback: Standardization / harmonization
- 2 Joint projects
  - Broad evaluation of many different tests (2001)
  - Specific evaluation of likely “candidates” (2003)

Specific evaluation study:

Ames TA98, UMU and Comet assay

Objectives:

- *(scientific)* Find most suitable genotox test for detecting mutagenicity in surface water
- *(for management)* Find cheaper alternative to Ames
- Define restrictions / limitations & suggest possible improvements

Description of UMU and Comet tests

UMU:

- Genetically modified Salmonella strain
  - specific gene incorporated in DNA, triggering enzyme production when DNA repair mechanism is activated (mutagenic substances)
  - Enzyme activity measured is proportional to conc of substance
- Well documented, highly standardized
- Applied in German WQ management (effluents), to be applied in NL

Comet:

- Lymph cells
  - Mutagenic activity may lead to broken DNA strands
  - DNA strands detected by electrophoresis of disrupted cells
  - Microscopic inspection of resulting “smear” (resembling a comet tail)

Logistics

- Five sampling campaigns
- Three locations (Rhine at German-Dutch border, Meuse at Belgian-Dutch border, and 1 Rhine intake site)
- Sample size 100 l
- Filtration, pH set to 7.0
- Adsorption on ion exchange resin (XAD-4)
- Elution with EtOH/cyclohexane (gradient)
- Drying, redissolution in EtOH at 25,000x

Expectations based on test characteristics

- Modes of action are different ⇒different responses
  - Ames: alteration of DNA basepair
  - UMU: induction of DNA repair mechanism
  - Comet: fragmentation of DNA strands
- Theoretical sensitivity: UMU≥Ames≥Comet
  - Fair level of mut. activity needed to produce enough DNA fragments for detection in Comet ⇒low sensitivity
  - Any mut. activity leading to damage, triggers UMU ⇒high sensitivity
  - Mut. activity has to induce base pair alteration to induce Ames regrowth ⇒intermediate sensitivity

Expectations not fully confirmed in experiment

- Ames results higher for Rhine than for Meuse (matches historic findings), highest response at Rhine border site
- Comet results similar pattern but less samples positive, highest response at intake
- UMU no clear distinction between Rhine and Meuse, number of positive samples between Ames and Comet
- Preconc factor UMU 750x vs Ames 25000x!

Object of current study:

- Comet shows highest response at intake site, fairly high at upstream border site
- Ames shows highest response at border site, fairly high at intake
- Does treatment reduce / remove this?
- Can this difference be explained?

Conclusions

- Results do not point to one single test to be used in WQMN
- Best combination:
  - 1 : Ames and Comet
  - 2 : UMU and Comet
- Management reaction:
  - Disappointed because results point to higher costs instead of expected savings…
  - nervous because of Comet findings at intake, helps funding of follow-up study…

Further information

www.riwa.org

- General description RIWA
- Publications (reports as pdf)